Standard Operating Procedure	SOP072

Title: USE AND MAINTENANCE OF THE NIKON ECLIPSE Ti MICROSCOPE AND CAMERA

Location: CBE Laboratory Unit

1. <u>PURPOSE</u>

This SOP describes the safe use and maintenance procedures for the Nikon Eclipse Ti Fluorescent Microscope and camera.

2. <u>SCOPE</u>

Operational procedures are described for the use of the Nikon Eclipse Ti fluorescent microscope in the dark room (H30).The microscope can be used for transmitted light observations (phase contrast, bright field) and red/blue, green/ultraviolet excited fluorescence observations of specimens. The procedure describes the process for switching between observations using a slider to image the cells cultured on glass or tissue culture treated plastic, cell suspension or cell aggregates and/or examine colour-stained or fluorescence cellular specimens. Specifically, this SOP describes procedures for capturing the images of the inspected specimens using an attached microscope digital camera system.

<u>RESPONSIBILITES</u>

3.1. The Operator shall:

(i)   Ensure that they are familiar with the equipment controls and requirements by reference to this SOP, the Operator and Maintenance Manuals, including operating instructions, record keeping and emergency shutdown procedures, and that they have completed training on microscope operation.

(ii)   Be responsible for the proper use and maintenance of the instrument as outlined in this document. Users must ensure that the working area is kept clean during work and disinfected after the work has been completed

3.2 The Responsible Person shall:

(i)   Schedule any service or preventative maintenance requirement with authorized service representatives. This must be coordinated with the Laboratory Manager. Ensure that the microscope has been suitably disinfected (see SOP 003) and a 'Decontamination Certificate' issued before external maintenance and servicing is performed.

(ii)   Record all maintenance, servicing and repair work and file with the microscope equipment file.

(iii)   Ensure that all operators have been given appropriate information, instruction, training and supervision in the correct use and maintenance of the microscope.

3. <u>EQUIPMENT AND MATERIALS</u>

- Nikon Eclipse Fluorescent microscope with lenses and phase contrast slider
- Fluorescence illuminator; provides fluorescence observation

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- Digital camera system and all adapters
- Neutral cleaning solution (e.g. 70% IMS)
- Microscope slides and cover slips

4. PROCEDURE

5.1. Operating Procedures for using the Microscope

5.1.1. Switching On

(i)   ALWAYS turn on both smart shutter boxes <u>before</u> turning on the microscope.

(ii)   To switch on the power box, press ignition for a few seconds until the "lamp ready" light shows.

(iii)   Ensure the microscope and all accessories are switched on <u>before</u> turning on the PC.

5.1.2. Workspace Overview

(i)   User rights: In a multi user working environment, the NIS Elements allows customised creation of user groups with varying rights to modify software and hardware settings.

(ii)   All Users from MS Windows accounts can then be loaded into NIS Elements and assigned to the desired group. The users can utilise the shared items but only those with permission can modify them – see below.

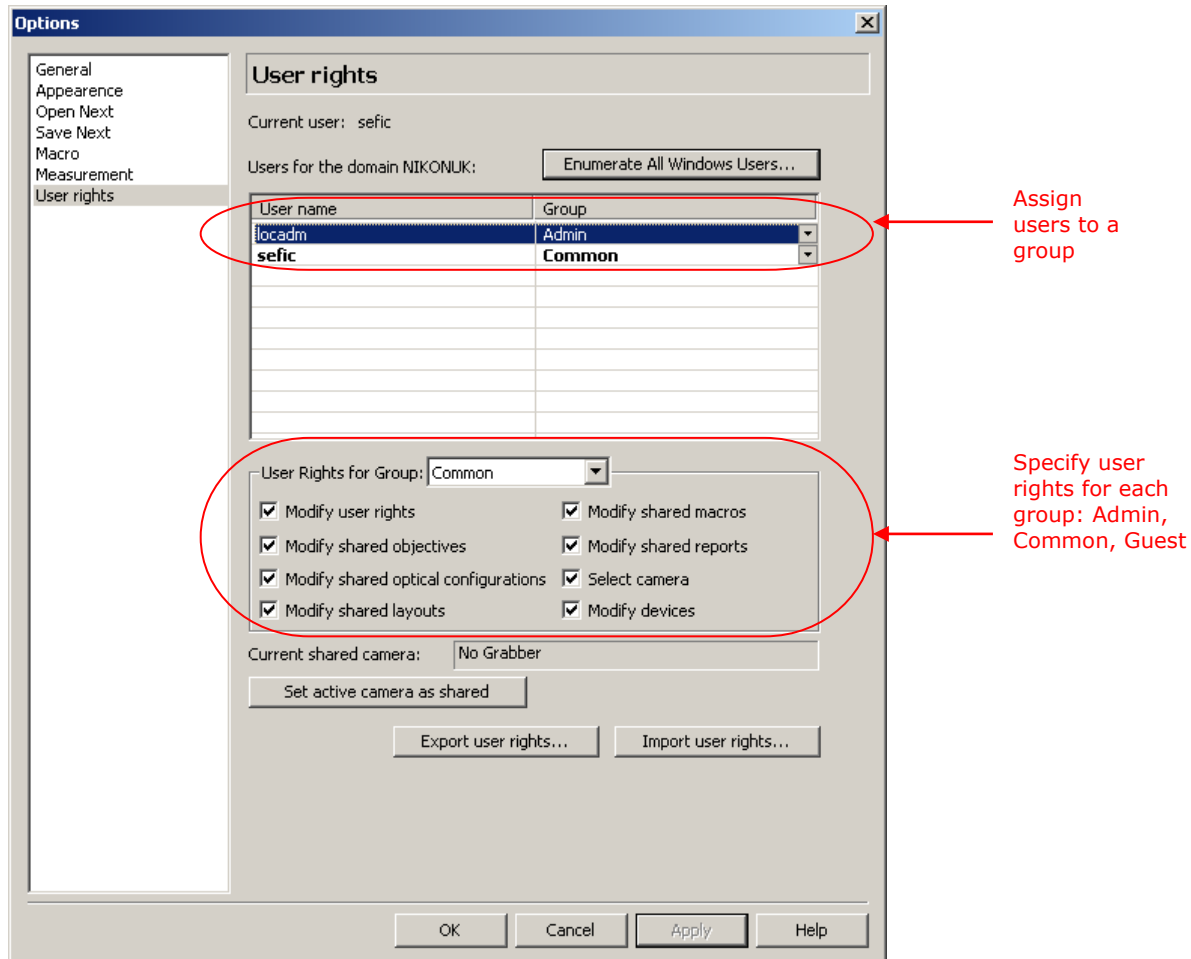(iii)   Run Edit > General Option and select *User Rights*

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5.1.3. Layout Manager

(i)   As standard, NIS Elements has 3 default layouts: Full Screen, Docked Controls and Measurements. Each layout can be customised with preferred selection of control windows in docking panes, toolbars, menu items. They can be set as Private or Shared. New layouts can be created.

(ii)   Create and position docking panes for Control windows by going to View > Docking Panes. Right click inside to select and open a control window from presented choices.

(iii)   You can access the Layout Manager from View > Layout or by right clicking on the layout tab shown below.

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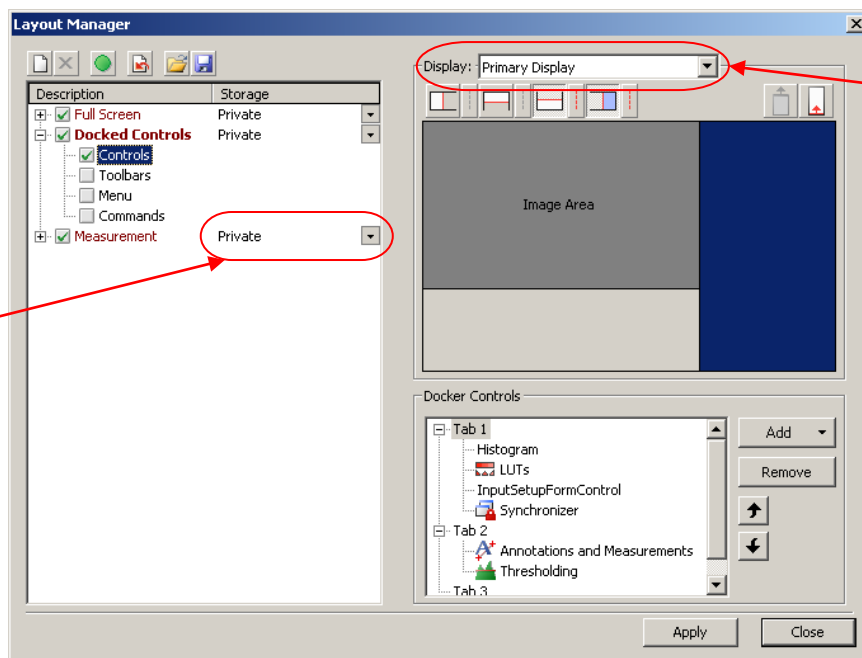
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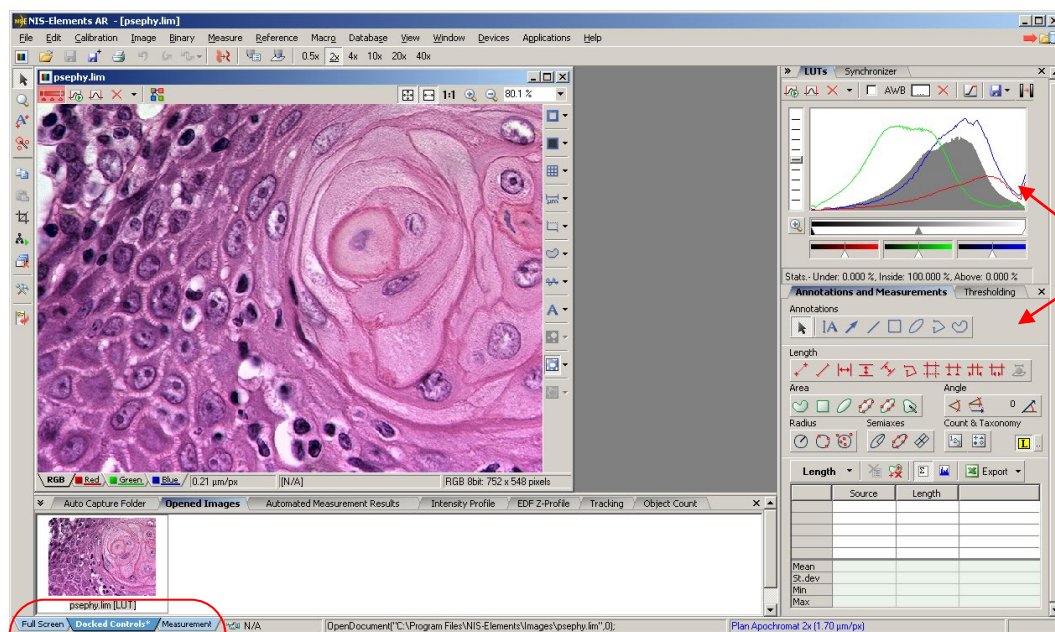
Control Windows in Docking panes

Default layouts

Storage can be set to be shared by other users but modified only if they have a permission to do it

Layouts can be customised over two display monitors

5.1.4. Access to Controls

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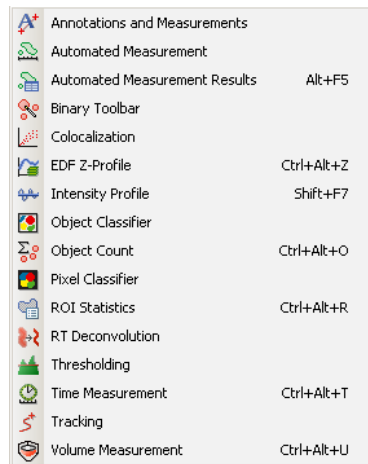
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(i)   Most of controls in NIS Elements can be found in View menu grouped in one of 4 major categories: Analysis, Acquisition, Visualisation and Macro.

(ii)   The choices available will be specific to the hardware components present in your microscope setup and software options in your licence.

Acquisition Control
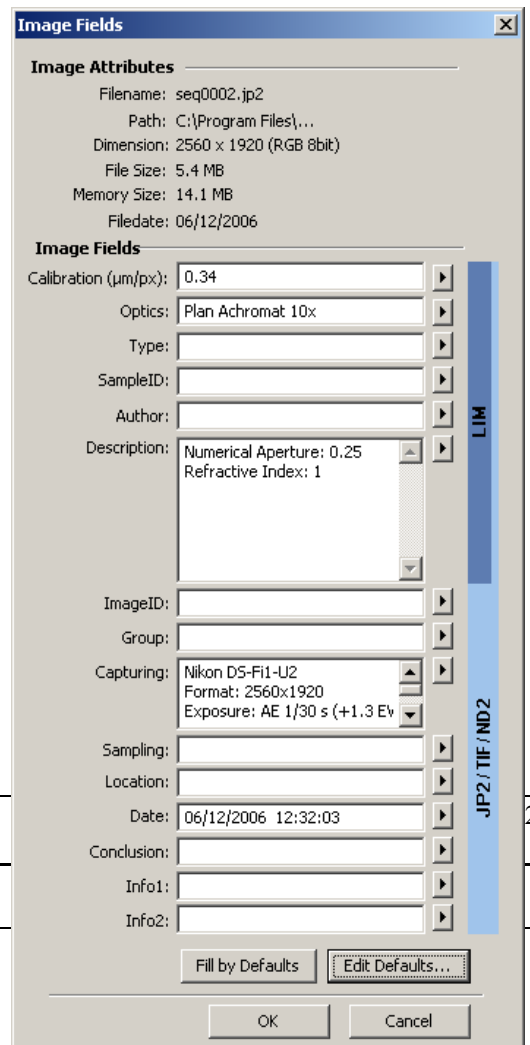Capture and Microscope control

Analysis Control
Annotations and measurement tools
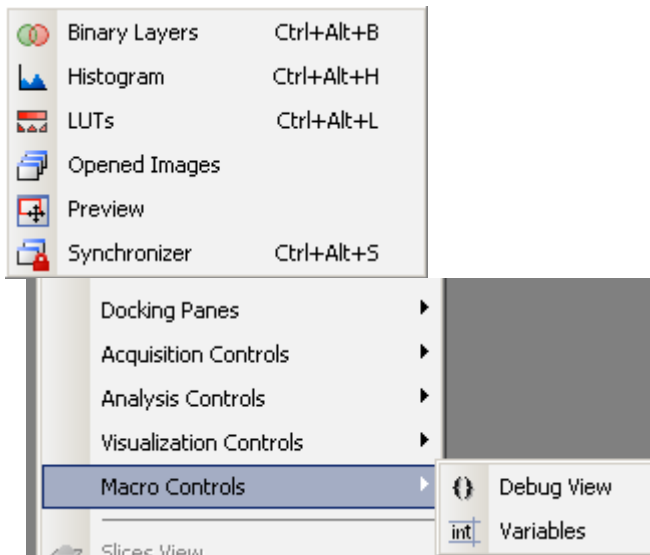
Visualisation Control
Macro Control

5.2. Image Document (File Formats, Image Properties)

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NIS-Elements software supports the following number of standard file formats.

- **JPEG2000 Format (JP2)** An advanced format with optional compression rates. Image calibration, text descriptions, and other meta-data can be saved together with the image.

- **Standard JPEG** files are used in many image processing applications.

- **Tagged Image File Format (TIFF)** This format can save the same amount of meta-data as JPEG2000. TIFF files are larger than JPEG2000 files but are loaded faster. TIFF files have several ways to store image data therefore there are many versions of TIFF. NIS-Elements support the most common TIFF modalities.

- **Windows Bitmap (BMP)** This is the standard Windows file format. This format does not include additional image description information such as author, sample, subject or calibration.

- **LIM Format (LIM)** Developed for the needs of laboratory image analysis package, nowadays all its features (and more) are provided by the JPEG 2000 format.

- **ICS/IDS sequences** are generated by some microscopes and consist of two files: the ICS file with information about the sequence; the IDS file containing the image data. The ICS file must be stored in the same directory together with the IDS file.

- **ND2 Format (ND2)** This is the special format for storing sequences of images acquired during ND experiments. It contains varied information about the hardware settings and the experiment conditions.

Images saved into JP2, TIF, ND2 or LIM will record and save image metafile information as shown left. Fields for additional user input information are available.

The **Image Properties** window is accessed by right clicking on the image.

5.3. Image Document (ND Files)

(i)   An ND document is a multidimensional image file. It may have one or more image sequences (z-stack, time-lapse, multichannel, multipoint) up to 6 dimensions (X, Y, Z, Time, fluorescence λ, Multipoint's). It is a result of an advanced image capture method or multidimensional experiment.

(ii)   Management of ND files such as cropping subsets can be found in File menu, ND submenu. You can crop out a single dimension or adjust start/end values from all dimensions present. The resulting crop is still an ND file format.

(iii)   ND files can also be exported into a series of tif or JP2000 images through File > ND > Export ND file function.
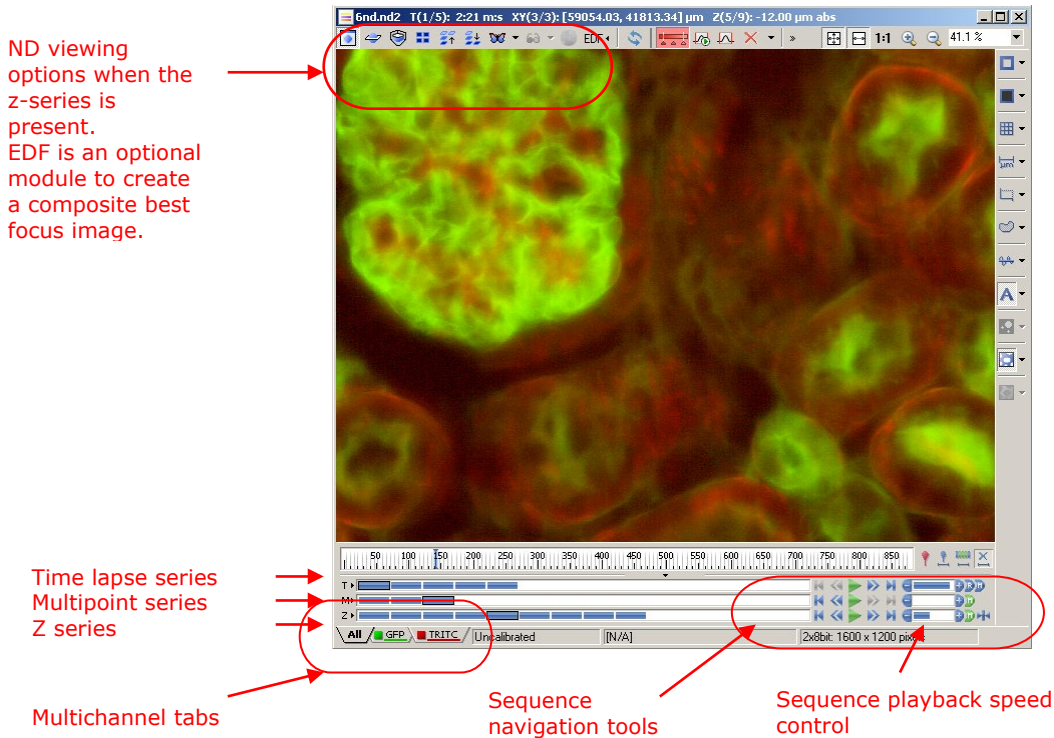
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ND viewing options when the z-series is present.
EDF is an optional module to create a composite best focus image.

Time lapse series
Multipoint series
Z series

Multichannel tabs

Sequence navigation tools

Sequence playback speed control

5.4 Camera Control

5.4.1. Camera Settings (DS-U2/L2 controller with DS-2M/5M/Fi1 camera head)

(i)   The camera settings allow control of camera parameters to set values like resolution, exposure mode (exposure time and camera gain), colour balance.

- **Resolution** setting will set the image size in pixels. Two resolution controls, Focus and Capture, set the resolution of the Live image and Captured image. The available values in drop-down boxes will vary depending on the camera head used (2 or 5 million pixels).
- **Exposure** mode can be set to Auto Exposure and Manual. Automatic mode will set the exposure time / gain automatically and the user will be able to finely adjust the brightness using AE Compensation scale (+/- 2EV). Manual mode requires the user to set his own exposure time / gain settings to achieve the desired image intensity.
- **Auto White** command will set the colour balance of the image. To do this, remove the specimen from the field of view and white balance the background.

(ii)   The colour balance depends on the microscope lamp voltage so once set the light levels should not be changed. Light intensity should be controlled via neutral density (ND) filters on the microscope instead.
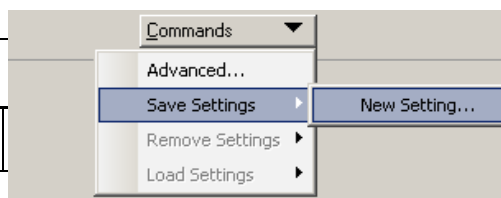
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In the Command drop-down options, Save Settings command allows users to create and store camera settings for different observation and capture methods.

5.4.2. Capture Controls

(i)   Start the live image window by pressing the green play button in the toolbar as shown below or you can press + on your keyboard

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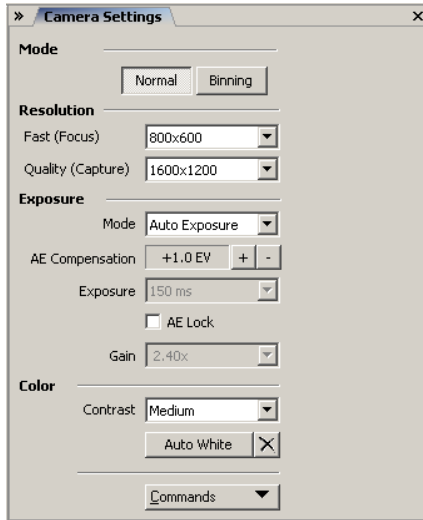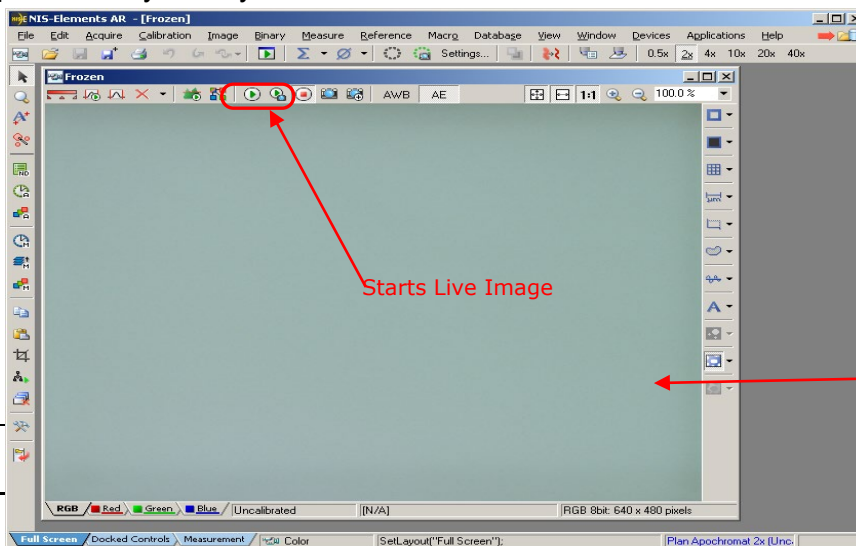
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(ii)   Use Camera settings to adjust the image resolution, exposure etc. The capture controls are found on the Live window toolbar: Live, Quality Live, Freeze, Capture and Autocapture.

(iii)   Capture will grab an image in a new window which needs to be saved manually in File > Save As.

(iv)   Auto capture uses the predefined location on your computer to capture and save with one click. The image prefix is set and images are saved sequentially by auto numbering into selected file format. Keyboard shortcut for Auto capture (Save Next function) is Enter key. Set the Auto capture properties in Edit > General Options, *Save Next* tab.
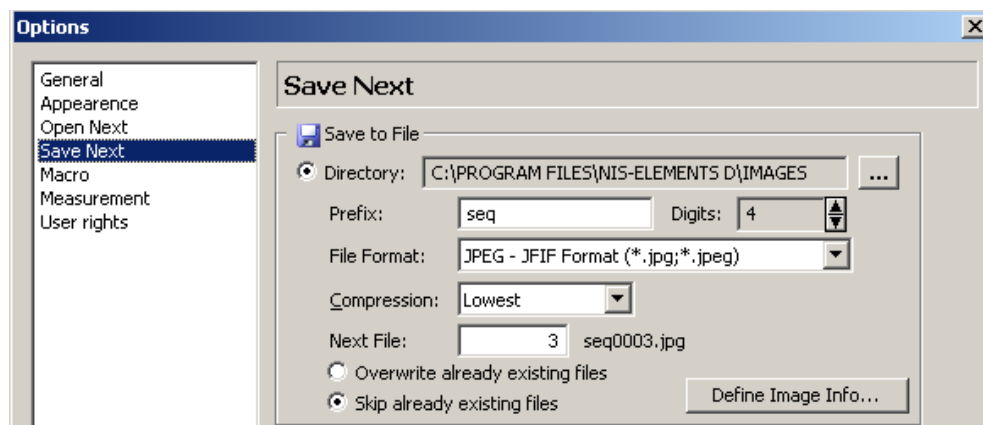
5.4.2.1. AVI Capture

AVI acquisition is an easy way of recording actions in real time. It produces *.avi video files, which can be played by any standard video player, so you do not need NIS-Elements to view the results.

(i)   Display the AVI Acquisition control window the [View > Acquisition Controls > AVI Acquisition] command.
(ii)   Adjust the advanced settings: define the file name and the destination folder.
(iii)   Click the [Record] button. The live image appears inside the application window and is being recorded according to the settings.
(iv)   Stop recording by the [Stop] button, or wait until the time defined in the [Duration] field passes.
(v)   When stopped, the AVI file remains opened in a new document window.

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Allows selection of compression codecs
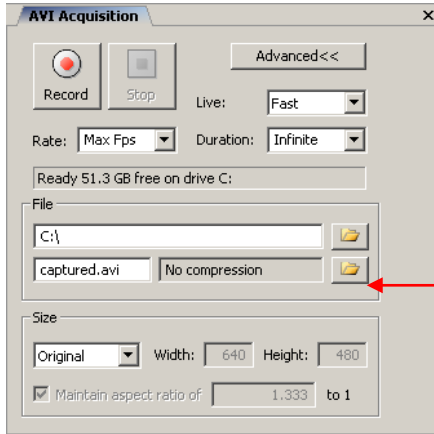
(vi) It is common to encode (compress) movies in order to save some disk capacity. This can be done using various types of codecs. Remember that the codec you choose must be available for the AVI playback.

(vii) *Video codec is a software tool which can [cod]e and [dec]ode video files. One of the following codec's should be used (FPS - maximum camera frame rate which can be handled, size - size of a reference 10s video, platform availability):*

- *Cinepack: FPS-1.1; size-1.2 MB; availability: Windows XP + Mac*
- *Intel Indeo: FPS-5.9; size-1.7 MB; availability: Windows XP*
- *Microsoft YUV: FPS-14.3; size-172 MB; availability: Windows XP*

5.5.2.2. RAM Capture

The Capturing-to-RAM technique enables user to record sequences displaying very quick actions. The quality and number of frames in a sequence depends on hardware capabilities of the computer system.

The technique uses a ring buffer to store temporary data. The ring buffer is a part of virtual memory that is being constantly (and repeatedly) filled with the live image data.

Turns Ring buffer On/Off

RAM Capture button

(i) Click on the *Settings* command next to the RAM capture buttons.

- Set the *Time buffered before/after* values. These represent the time before and after you click the RAM Capture button. The whole time interval will be included in the sequence.
- Confirm it by OK.

(ii) Turn the buffer ON by clicking on the leftmost button of the RAM capture tool bar to enable recording of Live image to RAM.

(iii) When the Camera is switched to Live mode, the left-side ring icon begins to indicate the buffer activity. If the RAM Capture button is pressed then, the captured sequence contains frames from the whole time interval before and after the button press. If you press the button while in the Frozen mode or without the time buffer ON, only the [Time buffered after] sequence is grabbed.

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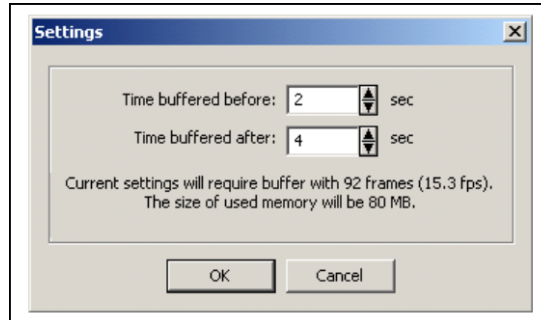| Written by: E. Ratcliffe	Reviewed by: P. Hourd	Approved by: R. I. Temple

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(iv)   To capture a sequence press the RAM Capture button.

(v)   A new document window containing the captured sequence opens.


5.5. Advanced Acquisition

5.5.1. Multi channel

(i)   In fluorescence microscopy it is often required to capture separate channels of multi labelled specimens and overlay them in a pseudo colour composite. This can be easily setup in NIS Elements providing the optical configurations already have the fluorescence filters setup. If you have a motorised microscope, the filter positions can be recorded and assigned to optical configurations with links to filter turret positions, shutter control etc.

(ii)   The next step is to configure the Multichannel Setup, Acquisition> Multichannel> Multichannel Setup

Check to save directly to a file on the computer

Check the wavelength box to create a name for and activate the channel
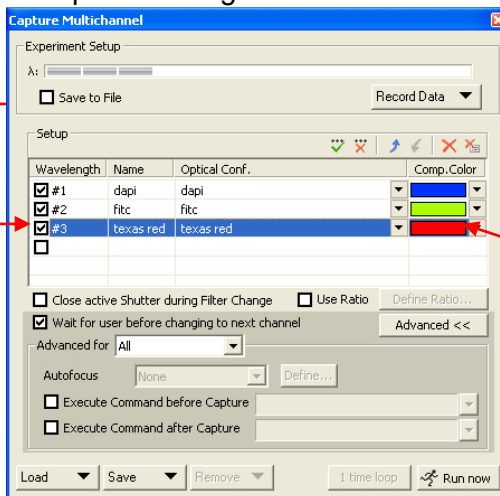
Assign the optical configuration from the drop-down list to the fluorescent channel and assign its colour

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(iii)   For motorised microscopes with automated fluorescence light path selection, the above Multichannel Setup enables the Automatic Multichannel acquisition of two or more channel. This function will create a new image document pre labelled with fluorescent channels used and drive the microscope and camera to create a multichannel colour composite image.
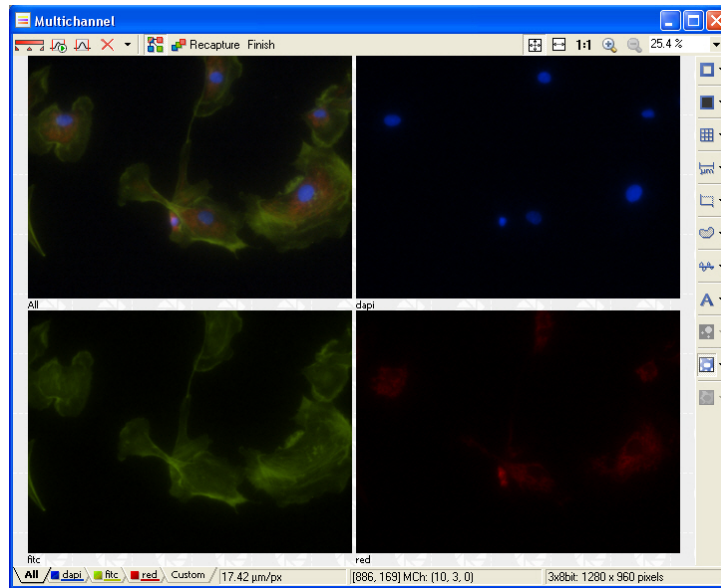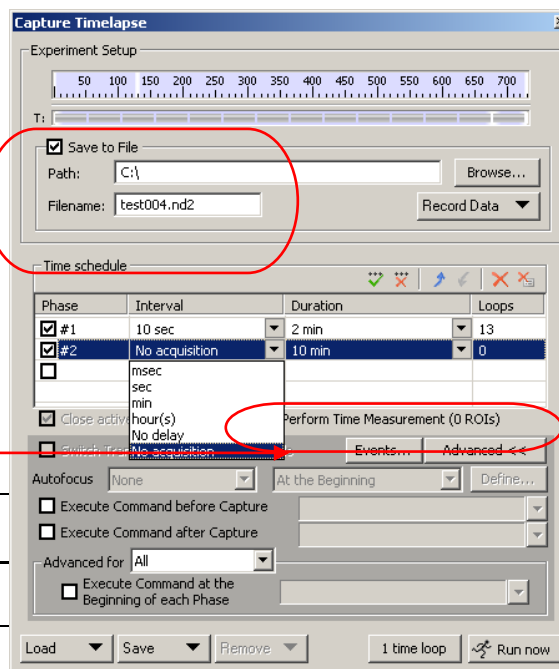

5.5.2. Time lapse

(i)   Time-lapse capture control allows users to set experimental conditions for capturing one or more time period (phases) which consist of three parameters: **interval** time between two consecutive image captures, total **duration** of the time phase and number of **loops** in the phase. As the three elements are linked together, setting two of them defines the third.

Check to save to an ND file as you capture otherwise images stored into system RAM and need to be saved afterwards

Check Perform Time measurement checkbox and define probes for average measurements (read more in Measurement notes section)

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(ii)   Tick the **phase** checkbox to define the time-lapse experiment. Interval time units are selected from the dropdown box. **No delay** captures images as fast as the camera settings and PC hardware are capable of achieving, **No acquisition** can be used for a time break with specified duration time in the next field.

(iii)   **Advanced** options allow setting of specific commands or macros before or after each capture and at the beginning of selected or all active phases.

(iv)   Time-lapse settings can be saved into a file and loaded for future experiments.

(v)   The result of a time series capture is an ND file, please refer to the *Image Document – ND files* in section 2 for more information.

5.5.3. Z stack

Z-stack acquisition will allow you to capture a sequence of images from different focal planes of the specimen.

(i)   The Z-series capture can be done through Acquire menu or, if your licence permits it, through Applications > Define/Run Experiment command.

(ii)   Z-stacks can be captured manually, frame by frame, by moving the focus through the sample in discreet steps. With the help of a motorised Z drive this acquisition is easy to setup and execute with a good precision.

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(iii)   **Top/bottom, Step:** Move the focus to top and click Top to record its position. Do the same for bottom. The recommended step size will be a function of the objective used and can be changed. Total number of slices is calculated and given in Steps field. Other method: define number of steps and step size is calculated.

- **Home position, symmetric range:** Record the home position and define the range value in microns.
- **Home position, asymmetric range:** Record the home position and define Below and Above ranges.

(iv)   If you have a motorised shutter you can close it between captures by checking the "Close active shutter…" checkbox.

(v)   Defined ranges with step sizes can be saved and loaded for future experiments.

(vi)   The result of a z-series capture is an ND file; please refer to the *Image Document – ND files* in section 2 for more information.

5.6. Optical Configurations

Optical configuration is an optical path setting which can include objectives, microscope settings and camera settings. They are managed in the Calibration> Optical Configurations window

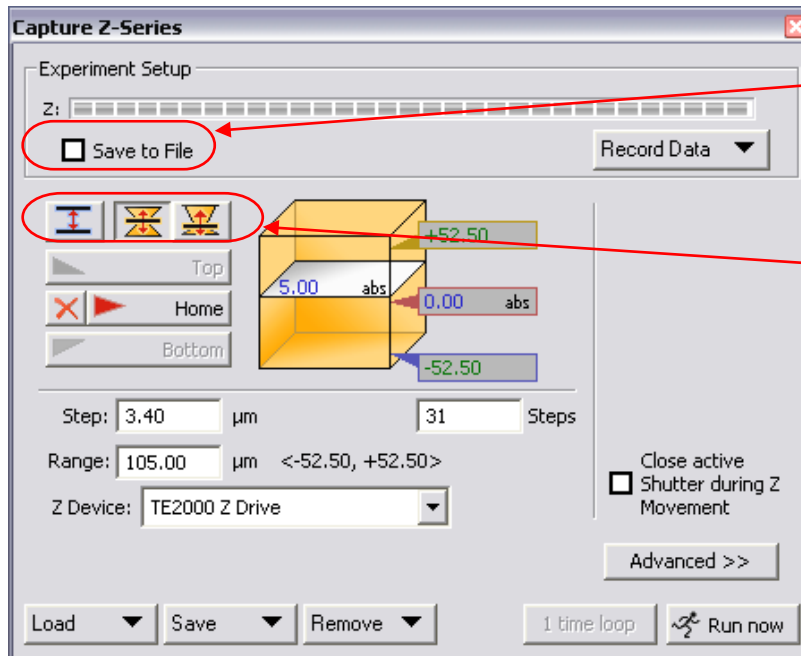| Version 002	Effective Date: 04/02/21	Review Date : 04/02/22
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(i)   This is an example of a lens configuration. Insert the objective in the Objective list, calibrate it using a graticule and create the configuration from the objective.


Optical configuration list may contain calibrated objectives and filter positions with an optional link to the camera settings. They appear as buttons on the main toolbar
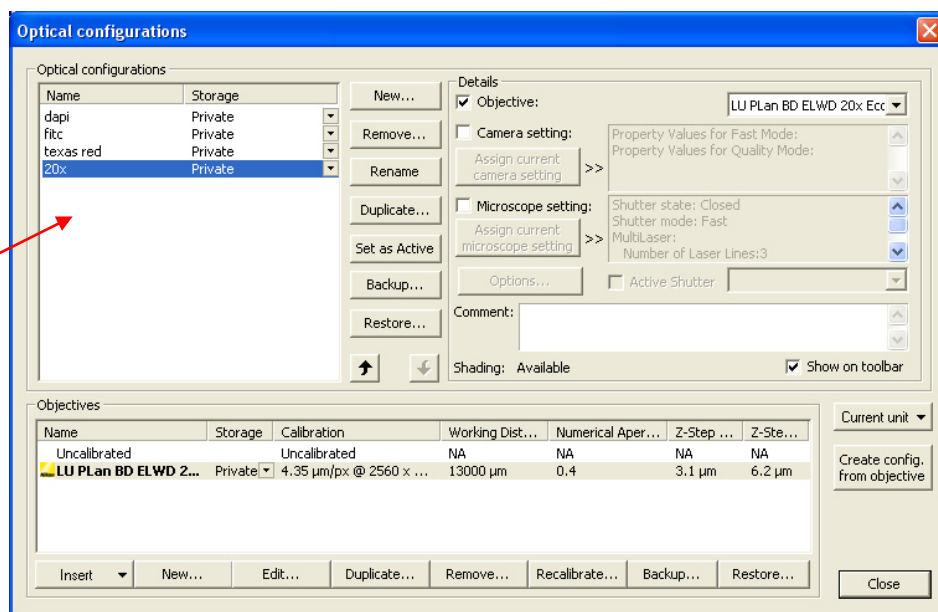
Create a list of objectives on your system by using the Insert command. Objectives are then calibrated with a graticule.

Create config from objective button will place it in the optical configuration list

(ii)   The following example shows a filter configuration. Click New to create and name the configuration, use the microscope control to place the filter in the optical path with other relevant settings and click the Assign current microscope setting to the highlighted filter optical configuration. You can also link the camera settings to the filter configuration.
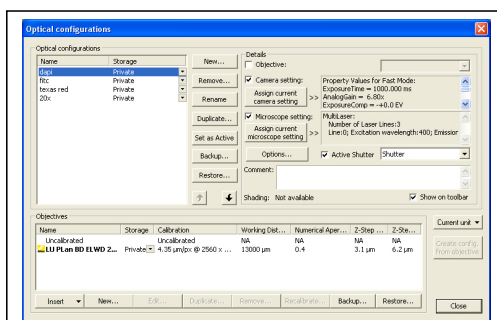
5.7. Microscope Control (Ti – E)

Motorised microscope components have their software control pads for an integrated system control. The Ti microscope pad looks like shown and is accessed in View>Acquisition Controls. The software and the
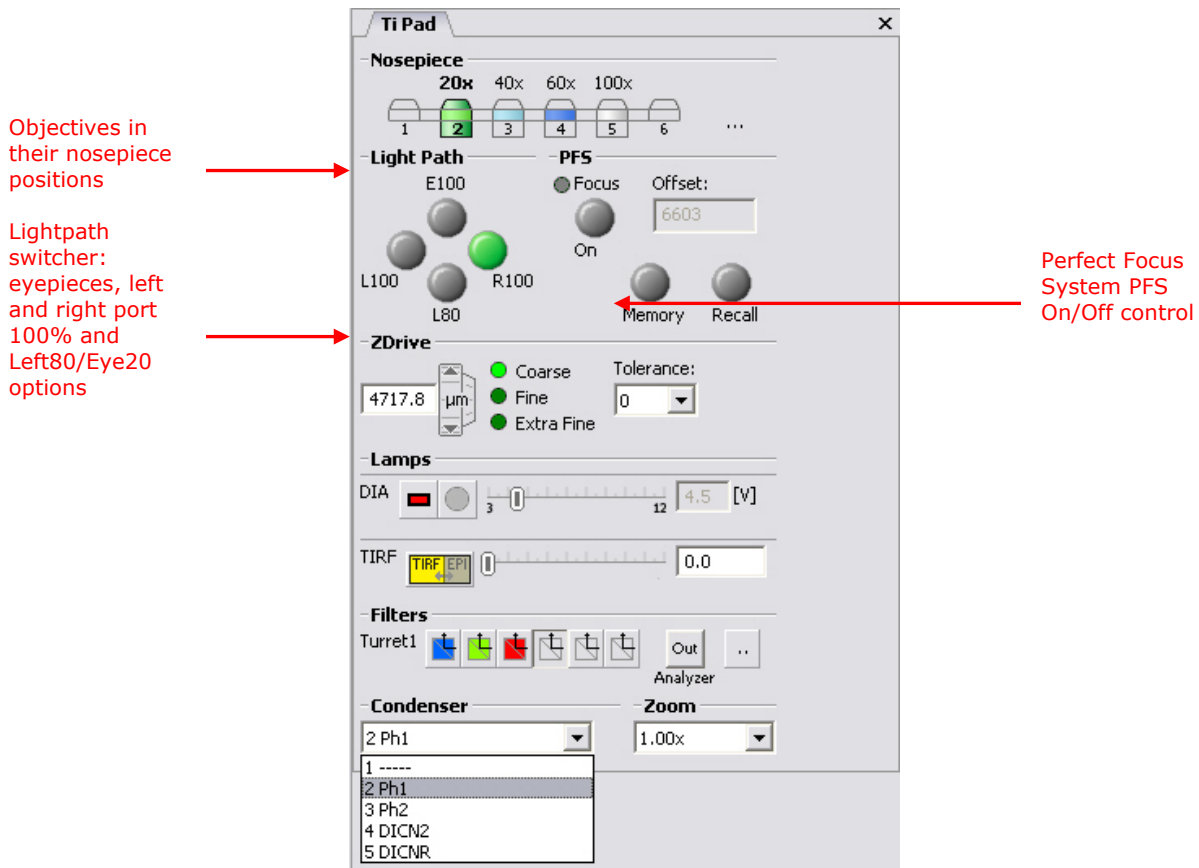
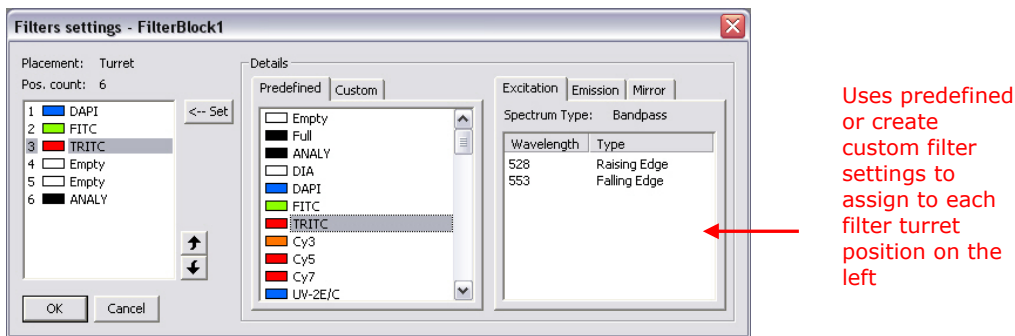**Standard Operating Procedure**   **SOP072**

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microscope have a two way communication: software commands drive the microscope and microscope positions are read by the software.

(i)   The filters in the filter turret are set by clicking the "…" button

(ii)   <u>Any microscope configuration can be saved by assigning it to an optical configuration. This makes setting complex configurations easy to use.</u>

5.8. Safe disposal of specimen

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Depending on the nature of the specimen, the user must dispose of it accordingly:

(i)   If the specimen contains any sharps then these should be disposed of in sharp containers as instructed by SOP003 "Disposal of Biological Waste".

          CAUTION: DO NOT place microscope slides/coverslips in autoclavable bags

(ii)   Biological waste generated through the usage of the microscope should be disposed of according to SOP003 "Disposal of Healthcare Waste".

(iii)   The use of Trypan Blue for cell counting will require for the Trypan Blue to be appropriately disposed of. As instructed by SOP029 "Safe handling and disposal of Trypan Blue", for small volumes (typically less than 50 uL of solution at 0.4%) of Trypan Blue retained under microscope cover slips can be washed into the sink followed by copious amounts of water. For further detail of disposal of Trypan Blue, please refer to SOP029 "Safe handling and disposal of Trypan Blue".

(iv)   Refer to appropriate COSHH, SOP039 and COP for other disposal of samples contaminated with other hazardous chemicals.

5.9. Preventative Maintenance Procedure

5.9.1 General Maintenance

(i)   If the lens becomes contaminated with any substance, contact the Responsible Person for instructions on required cleaning procedures.

(ii)   Clean all glass components by wiping gently with gauze. To remove fingerprints or oil smudges, wipe with gauze moistened with 70% IMS.

          CAUTION: Do not use organic solvents to clean microscope components other than the glass components (see below).

(iii)   Clean non-glass microscope components using a lint-free, soft cloth moistened with a diluted neutral detergent.

(iv)   Do not dissemble any part of the microscope. This could result in malfunction or reduced performance. **Do not disassemble or dismount any part of the system without consulting the Responsible Person.**

(v)   When not using the microscope, reduce the brightness, switch off both the main switch and the power supply, and keep it covered with a dust cover.

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(vi)   To minimise the interferences of blowing bulb to the microscope usage, two spare bulbs will be kept in the laboratory. Fill in the maintenance record and inform the Responsible Person if the spare bulb is used.

(vii)   The mercury burner has an average service life of 100 hours. The hour counter for the used life time is displayed on the power supply unit of fluorescence. When the hour counter on the power supply unit indicates 100hrs, replace the burner with a new one (for replacement instructions refer to Operator Instructions for Reflected Fluorescence System). Record in the Maintenance Log

5.10. Microscope Malfunction

(i)   The Responsible Person must be informed if any part of the equipment fails or malfunctions. With permission of the Responsible Person the user should consult the Operator Instruction Manuals for fault finding and troubleshooting procedures.

(ii)   All problems and corrective actions should be recorded in the maintenance log (Section 8). If the equipment fails to work or malfunctions and cannot be rectified according to troubleshooting procedures detailed in the Operator and Users Manuals the following should be observed:

- Inform the Responsible Person.
- Attach a "Do Not Use" notice.
- Contact the manufacturer for advice. External maintenance and servicing of the equipment can only be performed after it has been suitably disinfected (see SOP003) and a 'Decontamination Certificate' has been issued by the School/Building/Unit Safety Co-ordinator. An example of a Decontamination Certificate is given
- Ensure that the scheduling of any external servicing/maintenance is coordinated with the Laboratory Manager.

5.11. Useful Contacts:

- Kate Passam, Imaging Specialist, 07789968557, kate.passam@nikon.co.uk
- Alan Monk, Imaging Specialist, 07789968561, alan.monk@nikon.co.uk
- Diana Sefic-Svara, Software Service Engineer, 07876275271, diana.sefic-svara@nikon.co.uk

5. <u>DOCUMENTATION</u>

QS form - 009 Equipment decontamination certificate
QS form – 018 Preventative Maintenance, Inspection and service record

These forms can be found on the Loughborough University LEARN module for the Centre of Biological Engineering under QS-Form templates.

Written by: E. Ratcliffe	Reviewed by: P. Hourd	Approved by: R. I. Temple

Standard Operating Procedure   **SOP072**

Title: USE AND MAINTENANCE OF THE NIKON ECLIPSE Ti MICROSCOPE AND CAMERA

Location: CBE Laboratory Unit

SOP Version History

Version Reviewed	Date Revised/ Reviewed	Revision Summary	New Version Number
001	28/11/2012 K.Marrow	Moved to the lean template. Moved history to the back, removed references and health and safety and integrated them into the sop. No major written changes, except for minor editorial details. K.Marrow. FSOP`s created and stored on the CBE website. New issue number needed.	002
002	04/02/2021 K.Sikand	Minor changes. The microscope has been moved from H29 to H30 – changed location. Version number remains the same.	002

Version 002	Effective Date: 04/02/21	Review Date : 04/02/22

Written by: E. Ratcliffe	Reviewed by: P. Hourd	Approved by: R. I. Temple